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Phomopsis amaranthicola, a new species from *Amaranthus* sp.

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Abstract: In 1992, a pycnidial fungus isolated from diseased amaranth plants was found to be the causal agent of the observed stem and leaf blight. Inoculations performed in the greenhouse revealed that this organism caused a disease characterized by a general blight. The isolate was tentatively identified as a member of the genus *Phomopsis* and its morphological characteristics were compared to those reported in the literature for *Phomopsis* species collected from amaranth. The Florida isolate, ATCC 74226, was found to produce extremely large alpha conidia. Beta conidia were produced, as well as a third type of conidium of intermediate shape. Phylogenetic analysis, based on the sequences within the internal transcribed spacer regions of the ribosomal DNA, revealed that isolate ATCC 74226 differed substantially from the other *Phomopsis* species tested, including those that are known to produce the third type of conidium. Based on the morphological characteristics and DNA sequence information the isolate is described here as a new species of *Phomopsis*.

Key Words: Coelomycete, internal transcribed spacer region, ITS, pigweed

INTRODUCTION

Many plant species in the genus *Amaranthus* are troublesome weeds and are considered good targets for biological control. Surveys were conducted to seek potential agents for development as mycoherbicides. A pycnidial fungus isolated from diseased pigweed (*Amaranthus* sp.) was found to be the causal agent of a stem and leaf blight. Inoculations revealed that the disease caused by this organism began as leaf lesions, which expanded, coalesced, and moved to the leaf petiole, causing premature leaf abscission. Symptoms were observed within 5 d of inoculation. Symptoms then appeared on stems, with lesions girdling the stem, causing stem constriction and toppling of plants. The severity of the symptoms observed in the field and then reproduced in subsequent inoculations warranted further investigation of the fungus as a potential biological control agent for *Amaranthus* spp.

Approximately 65 species of *Phomopsis* are plant pathogens, of which 30 are found in the United States. The identification of species in this genus, based primarily on morphological characteristics, is hindered by plasticity of characters. Difficulty with identification is also compounded due to teleomorphic associations with *Diaporthe* spp. being known for only about 20% of the named *Phomopsis* species. Evidence that host association is inadequate for speciation in this genus was provided by Brayford (1990), who showed that *Phomopsis* occurring on twigs and bark of *Ulmus* L. in the United Kingdom and other parts of Europe belonged to different morphological and genetic groups. Both groups were isolated from a variety of tree species, showing that more than one species could be found on a single host and more than one host could support a single species.

Species of *Phomopsis* characteristically produce two types of conidia, referred to as α conidia and β conidia. Three species reported to have a third type of conidium, the C conidium, are *P. hordei* Punith., *P. oryzae* Punith., and *P. phyllanthi* Punith. These species were isolated from *Hordeum vulgare* L., *Oryza sativa* L., and *Phyllanthus* L. spp., respectively (Punithalingam 1975). A single species of *Phomopsis* (Sacc.) Bubák has been reported to infect amaranth. This species, *P. amaranthi* Ubriszy & Vörös was reported from Hungary from *Amaranthus retroflexus* L. (Ubriszy and Vörös 1966).

Rehner and Uecker (1994) tested the relationship

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TABLE I. Descriptions of cultures used for comparison of the internal transcribed spacer regions.[a]

Isolate	Host genus	Location	Conidial types
75[b]	*Amaranthus*	USA—Iowa	α (AF079770)[j]
BG	*Amaranthus*	USA—Florida	α (AF079771)
FP1	*Amaranthus*	USA—Florida	α (AF079772)
FP3	*Amaranthus*	USA—Florida	α (AF079773)
PHO[c]	*Glycine*		N/A (AF079775)
Ma[d]	*Amaranthus*	USA—Arkansas	N/A (AF079774)
B[i]	*Amaranthus*	USA—Florida	α, β, and C (AF079776)
Po[e]	*Oryza*		α, β, and C (AF079777)
624[f]	*Glycine*	USA—Florida	Unknown
537[f]	*Kalmia*	USA—New Jersey	α and β
528[f]	*Magnifera*	Puerto Rico	α and β
522[f]	*Sassafras*	USA—New Jersey	Unknown
512[f]	*Juniperus*	USA—New Jersey	α and β
476[g]	*Vaccinium*	USA—New Jersey	α and β
468[g]	*Vaccinium*	USA—New Jersey	α and β
649[f]	*Convolvulus*	Canada	α
642[f]	*Glycine*	USA—Maryland	α
597[f]	*Solanum*	Dominican Republic	Unknown
484[g]	*Capsicum*	USA—Maryland	α and β
456[g]	*Stokesia*	USA—Mississippi	α and β
452[g]	*Capsicum*	USA—Texas	α and β
gm2[h]	*Prunus*	USA—Georgia	α and β
GAP8[h]	*Prunus*	USA—Georgia	α and β
GLB06[h]	*Prunus*	USA—Georgia	α and β

[a] All sequences for isolates with labels composed of a three digit number were obtained from GenBank and were deposited by S. Rehner and F. Uecker. Sequences for isolates gm2, GAP08, and GLB06 were also obtained from the GenBank. These isolates were deposited by W. Uddin (1996).

[b] Isolate provided by C. Block, North Central Regional Plant Introduction Station, Ames, Iowa.

[c] *Phoma medicaginis,* ATCC 52798.

[d] Isolate of *Microsphaeropsis amaranthi* provided by G. Weidemann, University of Arkansas.

[e] *Phomopsis oryzae,* IMI 158929.

[f] As reported in Uecker 1988.

[g] As obtained from the personal notebooks of F. Uecker, courtesy of R. Pardo-Schultheiss, USDA, Beltsville, NM.

[h] Isolate information from W. Uddin 1997.

[i] Described herein as *Phomopsis amaranthicola.*

[j] GenBank accession number of new deposits

of *Phomopsis* spp. with host association through sequencing of the internal transcribed spacer (ITS) regions of the ribosomal DNA. Their work resulted in three groups based on the ITS phylogeny. Group A consisted of two subclades. High similarity of one subclade, referred to as A1, if viewed as a single species, could be indicative of a broad host range, including plants belonging to the genera *Paulownia* Siebold & Zucc., *Epigaea* L., *Kalmia* L., *Cornus* L., *Tsuga* Carriére, *Lindera* Thunb., *Vaccinium* L., and *Picea* Dietr., to name a few. Members of the second subclade, A2, were all associated with cultivated *Vaccinium* spp. Group B, with the exception of a single isolate, originated from southern temperate to tropical regions and produced elongate paraphyses in the conidiomata. Isolates in Group C were obtained from a wide range of herbaceous cultivated plants. Results of this study suggest that there is a great deal of variation within the genus that could be attributed to geographical distribution.

MATERIALS AND METHODS

Isolates.—Single-spore isolates were recovered from agar slants and soil tubes by plating onto Difco potato dextrose agar (PDA). A single isolate, ATCC 74226 (isolate B, TABLE I), having consistent growth in all subcultures, was used for determination of morphological characters. Plate cultures were inoculated using a 5-mm^3 mycelial plug taken from 10-d-old PDA cultures. These were then incubated at 25 ± 2 C with a 12-h light cycle. Conidia were harvested from plates using 10 mL of sterile deionized water per plate and gentle scraping with a rubber policeman. A hemacytometer was used to count conidia. Data obtained included proportions of each type of conidium and measurements of conid-

a. Conidial germination was evaluated by placing a thin coating of PDA onto a sterile glass slide. A droplet of a conidial suspension, obtained from plates in the same manner as above, was placed onto the PDA and a sterilized cover slip was placed on top. The slides were allowed to incubate for 20–24 h on a bench top and the proportions of germinating conidia were pooled with the spore germination data obtained directly from the suspensions.

The dimensions of pycnidia were measured at the first sign of sporulation. Measurements were also made of conidia and pycnidia taken from inoculated *A. hybridus* L. tissue that was allowed to senesce and dry slightly to induce pycnidial formation, as had been observed in the field. Stem pieces with pycnidia were placed in moist chambers to induce sporulation. In an attempt to induce a perithecial state, infected tissue samples were allowed to dry and were stored in sand or soil, at both 4 C and at 30 C, in the absence of light. The available isolates were also paired on PDA and V-8 agar plates (Miller 1955).

The physical dimensions of conidia were measured using cultures of the ATCC 74226 isolate grown on PDA and from diseased plant tissue. Samples were obtained from 1- to 2-wk-old colonies from plates that were incubated at 25 ± 2 C. Four samples of conidial droplets from each of five replicate cultures were used. Droplets were mounted on glass slides in water and conidia were measured in two randomly chosen fields until 100 measurements of each type of conidium were taken.

Isolate ATCC 74226 was grown on artificial growth media, including Difco potato dextrose agar (PDA), V-8 agar, Difco (Detroit, Michigan), Difco corn meal agar (CMA), Difco oatmeal agar (OMA), oatmeal agar (OMAII), and amaranth-infusion agar, with (AIAS) and without sucrose (AIA), for comparison of growth characteristics. Amaranth infusion agars were prepared by boiling 50 g of amaranth leaves and stems in 500 mL of deionized water for 30 min. The strained extract (200 mL) was added to 800 mL of deionized water containing 14 g of agar (Fisher Scientific, Pittsburgh, Pennsylvania). Sucrose was then added to a portion of the medium to achieve a concentration of 2%. Oatmeal agar was prepared using 60 g/L of oat flakes and 14 g/L agar. Growth rate of the isolate was recorded daily.

The following isolates, obtained from symptomatic tissues of accessions of *Amaranthus* spp. were compared for growth of mycelium and spore production: 75A, 72A, and 72B provided by Dr. Charles Block of the North Central Regional Plant Introduction Station, Ames, Iowa; BG from Belle Glade, Florida; FP1 and FP3 from Fort Pierce, Florida; AI from Immokalee, Florida; and BRZ from Jaboticabal, Sao Paulo, Brazil. Isolates 75A, 72A, and 72B were tentatively identified by Dr. Block as members of the genus *Phomopsis*. Each isolate was transferred onto six petri plates of each medium. Plates were inoculated using a 5-mm diam mycelial plug from cultures grown on PDA.

DNA analysis.—The following isolates were grown in liquid potato-dextrose broth (PDB) shake cultures: B (ATCC 74226) from Gainesville, Florida; FP1 and FP3 from Fort Pierce, Florida; 75A from Ames, Iowa; MA *Microsphaeropsis amaranthi* from Arkansas; Pho, *Phoma medicaginis* Malbr. & Roum. (ATCC 52798); and PO, *Phomopsis oryzae* (IMI 158929).

Flasks (250 mL) containing 50 mL of PDB were inoculated with three, 5-mm diam mycelial plugs from 10-d-old PDA cultures of each of the isolates derived from single-spore cultures stored at 9 C. After 7–10 d of growth in shake culture, the contents of the flasks were filtered through sterile cheesecloth, squeezed dry, and rinsed three times with sterile deionized water. Mycelium was then placed into 13-mL plastic tubes, stored for 24 h in a −80 C freezer, and lyophilized for 24–48 h. The dry mycelium was then mixed with liquid nitrogen, ground to a fine powder, and DNA was extracted using the method of Koenig (1997).

The crude pellet was dissolved in TE buffer containing 2M NaCl and reprecipitated in two volumes of ethanol. The DNA was then pelleted and the tubes were allowed to dry. The pellet was resuspended in 100 μL of TE buffer (10 mM Tris, 1 mM EDTA). Samples were then placed at 4 C until the DNA was dissolved.

Approximately 100 ng of template DNA per 100 μL reaction mixture were used in symmetric PCR. The internal transcribed spacer regions (ITS) of the nuclear ribosomal repeat were analyzed using primers ITS4 and ITS5 (White et al 1990, Bruns et al 1991). Primers were synthesized at the University of Florida Interdisciplinary Center for Biotechnology Research Oligonucleotide Synthesis Laboratory (Gainesville, Florida). Polymerase chain reactions were performed using final concentrations of the components in the reaction mixture as follows: 20 mM Tris-HCl (pH 8.4); 50 mM KCl; 1.5 mM $MgCl_2$; 1 μM of each primer; 200 μM each of dATP, dCTP, dGTP, and dTTP; and 2.5 U of Taq polymerase (Gibco-BRL, Gaithersburg, Maryland) per 100 μL of reaction mixture. A GenAmp 6000 (Perkin-Elmer Applied Biosystems, Foster City, California) was used for the amplification. The cycling conditions used included an initial denaturation step of 2 min at 94 C, with 32 cycles of 94 C for 45 s, 55 C for 30 s, and 72 C for 45 s each. The last cycle included a 10-min incubation at 72 C and then storage at 4 C. Unincorporated nucleotides and primers were separated from double stranded PCR products using Wizard PCR Preps (Promega Inc., Madison, Wisconsin) according to the manufacturer's instructions. Sequences, provided by the University of Florida Interdisciplinary Center for Biotechnology Research DNA Sequencing Laboratory, were aligned manually. Sequences were deposited with GenBank (AF079770–AF079777). Initially, sequences from both ITS1 and ITS2 were aligned as a single data set (TreeBASE SN254). Alignment of these sequences resulted in a data matrix of 498 sites in 24 isolates. Alignment of both ITS1 and ITS2 required the insertion of gaps to maximize sequence similarity. Because of ambiguities in alignment, short sequence segments were excluded from the analysis. Phylogenetic analyses were performed using PAUP version 3.1.1 (Swofford 1993). The positions in the alignment were used as uniformly weighted characters; single gaps were treated as missing characters; and regions that could not be aligned or regions with large, continuous gaps were excluded from the analysis. The analysis was based on 254 informative characters. *Microsphaeropsis amaranthi* was used as the outgroup. The analysis included 24 sequences, 13 cho-

sen from those deposited in GenBank by Rehner and Uecker (1994), and three deposited by Uddin (1996), which were chosen based on a wide range of host associations (TABLE I). The analysis was performed using 1000 replicate heuristic searches with random sequence addition. Support for groupings was determined by bootstrapping of 500 replicate data sets, unless noted, using random input of sequences and by determining the decay indices using TreeRot (Sorenson 1996).

RESULTS

Phomopsis amaranthicola Rosskopf, Charudattan, Shabana, et Benny, sp. nov. FIGS. 1–5

Coloniae in agaro "potato dextrose" albidae, floccosae, denique fumosus. Mycelio septalis, ramosa. Pycnidia abundanti, producens concentricus 0.5-cm separatus, solitaria. Pycnidia globosa, ostiolata et unilocularia, 371–287 μm, exudato sporali dilutus armeniacus. Paries pycnidialis brunneis vel piceus, cellulis multistratosis, eustromaticus. Conidiophores hyalinae, simplicia, recta vel ramosa. Cellulae conidiogenae hyalinae, procreans α conidia multilocus, phialidicae, exorientes stratis ex intimis cellularum cavitatis pycnidii. Alpha conidia hyalina, fusiforme-elliptica, 1–7 guttulata, unicellularia, 6.6–24.0 × 2.2–6.6 μm, average 14.1 × 5.7 μm (mode 13.2 × 6.6 μm). Beta conidia hyalina, filiformia, hamatus vel recta, 24.2–28.6 × 1.1–2.2 μm, average 27.8 × 1.6 μm (mode 28.6 × 1.1 μm). C conidia sparsa, hyalina, variabilis guttulata, 18.0–22.0 μm, average 19.9 × 2.4 (mode 18.2 × 2.6). Pycnidia in caules emortuis immersa, erumpescentia, singularis, unilocularia.

Colonies produced on potato dextrose agar white, floccose, turning gray to brown. Mycelium septate and branched. Pycnidia abundant, produced in concentric rings 0.5-cm separating, solitary. Pycnidia globose, ostiolate, and unilocular, 371–287 μm, with conidia released in a peach-colored matrix. Pycnidial wall brown to black and multicelled. Conidiophores hyaline, occasionally branched. Conidiogenous cells hyaline, having multiple loci of alpha conidium production, phialidic, lining the inner-wall of the cavity. Alpha conidia hyaline, fusiform-elliptic, containing 1–7 guttules, aseptate, 6.6–24.0 × 2.2–6.6 μm, $\bar{x}$ = 14.1 × 5.7 μm (mode 13.2 × 6.6 μm). Beta conidia hyaline, filiform, hamate or straight, 24.2–28.6 × 1.1–2.2 μm, $\bar{x}$ = 27.8 × 1.6 μm (mode 28.6 × 1.1). C conidia sparse, hyaline, guttulate, 18.0–22.0 μm, $\bar{x}$ = 19.9 × 2.4 (mode 18.2 × 2.6). Pycnidia on dead stems immersed and erumpent, single, and unilocular.

HOLOTYPE. USA. FLORIDA: Alachua County, Gainesville, on *Amaranthus hybridus,* 7 Oct 1998, *E. N. Rosskopf FLAS F56785* (Mycological Herbarium, University of Florida, Gainesville, Florida). ATCC 74226.

Symptoms produced on *Amaranthus hybridus* that were spray-inoculated with conidial suspensions of Florida isolate B consisted of round to elliptical lesions with either tan or light brown centers surrounded by red-brown rings (FIG. 1A). Coalescing, large lesions developing on leaf petioles and stems caused a stem and leaf blight (FIG. 1B), which led to premature defoliation, girdling of stems, and plant mortality. Leaf symptoms were apparent 5–7 d after incoulation. Stem lesions developed within 2 wk of inoculation. Occurrence of plant mortality was variable (data not shown).

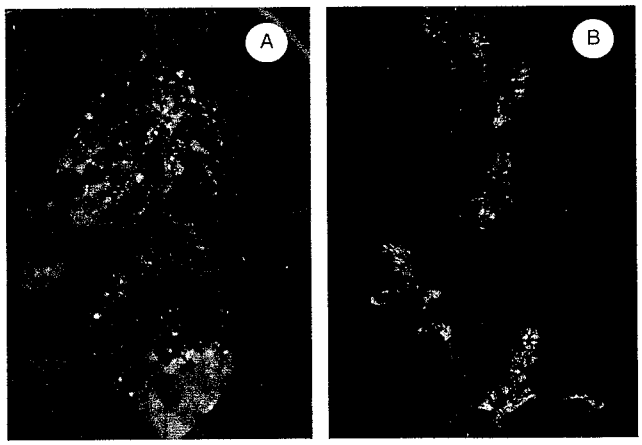
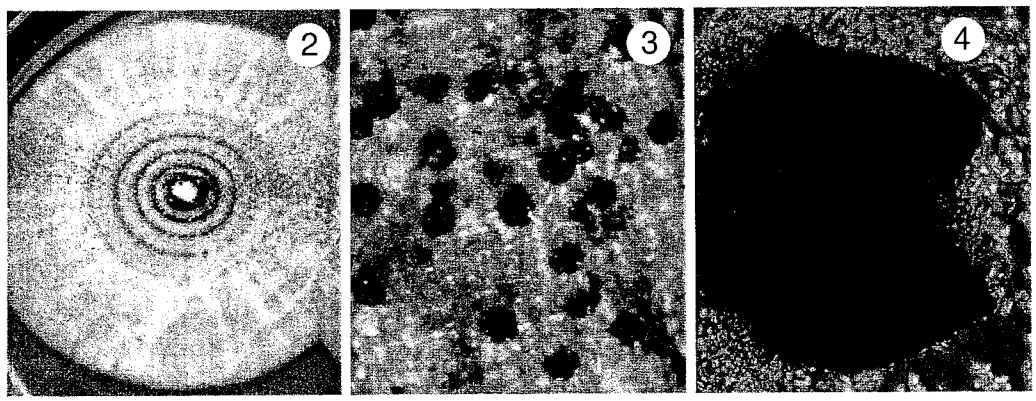


FIG. 1. A. Leaf lesions on *Amaranthus hybridus* L. resulting from inoculation with *Phomopsis amaranthicola.* B. Stem lesions on *Amaranthus hybridus* L. resulting from inoculation with *Phomopsis amaranthicola.*

Growth on PDA and all other media was initiated with white mycelium growing in a circular pattern. Pycnidia were produced within 14 d after culture initiation, beginning closest to the point of inoculation and formed in concentric rings approximately 5-mm apart (FIG. 2). Pycnidia produced on all types of media, as well as those produced on plant material, were ostiolate (FIGS. 3, 4). There were no significant differences between pycnidia produced on agar and natural substrate.

Alpha, β, and C conidia (FIGS. 5A–C) were produced within the same pycnidium when all spore types were present. In pycnidia containing all three types of conidia, an average of 87% of all conidia produced were α conidia. Beta conidia contributed 10% and C conidia an average of 3% to the total conidial counts. Conidia were produced on hyaline, phialidic conidiophores, which were occasionally branched (FIGS. 5D, E). Alpha conidia were occasionally produced from multiple loci of conidiogenesis within the conidiophore.

Suspensions containing mixtures of the conidial types were plated on water agar in order to obtain single conidial isolates and examine the germination of conidia. Germination of conidia on PDA-covered glass slides produced the following results. No C conidia were observed to germinate, although they did



FIGS. 2–4. Growth and sporulation of *Phomopsis amaranthicola*. 2. White mycelium with dark pycnidia produced in concentric rings around the point of inoculation on potato dextrose agar (×1). 3. Pycnidia on a stem section of an inoculated *A. hybridus* plant. Stem sections were placed on wetted Whatman 3 filter paper and placed in a moist chamber until sporulation (×10). 4. Sporulating pycnidium in a squash mount (×600).

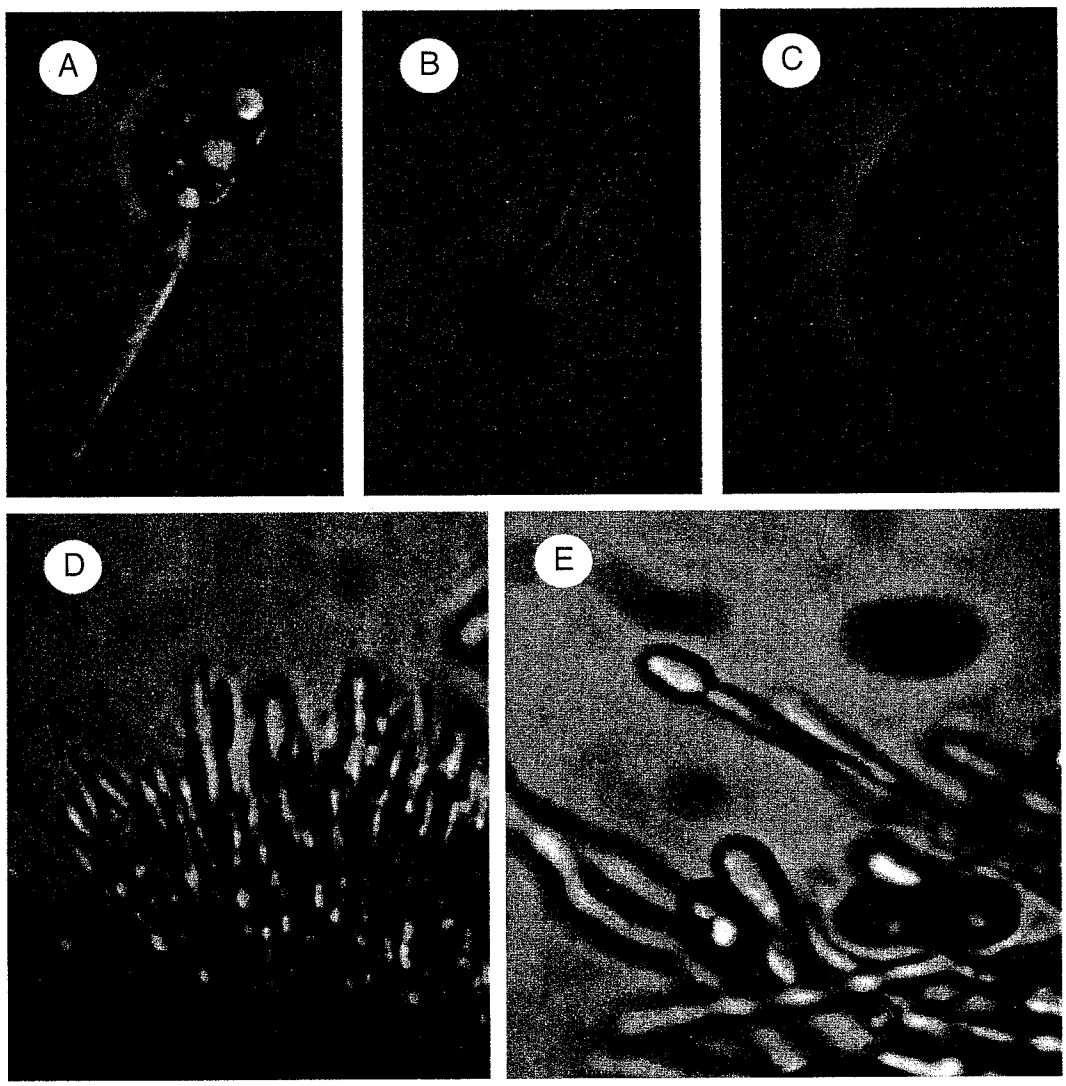


FIG. 5. Conidia and condiophores of *Phomopsis amaranthicola*. A. α conidium observed by light microscopy (×6000). Note guttules within the conidium. B. β conidium (×6000). C. C conidia (×6000). D. Conidiophores with α conidia (×2400). E. Conidiophore with α conidium (×6000).

TABLE II. Comparison of morphological characteristics of *Phomopsis amaranthi* and *P. amaranthicola*

Phomopsis species	Statistic	Pycnidia (μm)	Alpha conidia (μm)	Beta conidia (μm)	C conidia (μm)
P. amaranthi	Range	300–600 × 200–300	6.7–9.0 × 2.7–3.6	18–27 × 0.5–1.5	None present
P. amaranthicola	Range	168–762 × 148–584	6.6–24.0 × 2.2–6.6	24.2–28.6 × 1.1–2.2	18.0–22.0 × 2.0–3.1
	Average[a]	371 × 287	14.1 × 5.7	27.8 × 1.6	19.9 × 2.4
	Mode[b]	297 × 198	13.2 × 6.6	28.6 × 1.1	18.2 × 2.6

[a] Averages for *P. amaranthicola* obtained from material obtained from cultures and infected plant material.
[b] Mode for *P. amaranthicola* obtained from material obtained from cultures and infected plant material.

exhibit incipient morphological changes associated with germination, including irregular swellings. After more than 20 h, less than 30% of β conidia showed morphological changes and production of thread-like mycelium. This mycelial growth, like that reported for *P. helianthe* (Muntanola-Cvetkovic et al 1985), disintegrated and did not produce colonies. Alpha conidia were found to germinate approximately 87% of the time using this method.

Measurements of conidia and conidiomata were obtained for the Florida amaranth isolate B (ATCC 74226) from PDA (TABLE II) with the comparative measures reported for *Phomopsis amaranthi* from amaranth in Hungary. The average size of α conidia of the Florida isolate are larger than the range reported for *P. amaranthi.* In addition to the size differences in both α conidia and β conidia of both isolates, there is the marked absence of the third type of conidium from the description of the Hungarian isolate.

Pairing of isolates on PDA and V-8 juice agar did not result in the production of a sexual stage, nor did there appear to be any zonation. Instead, all of the paired isolates grew in an intermingled fashion. Infected stem pieces stored in soil and sand and exposed to temperatures of 4 C and 30 C did not produce perithecia. Removal of these tissues and subsequent plating resulted in regrowth of *P. amaranthicola* from all pieces.

The Florida isolate (ATCC 74226) grew more slowly than any of the other test isolates on all of the media tested (data not shown). The isolate of *P. oryzae* and the Florida isolates were the only isolates that produced α, β, and C conidia. Isolate BRZ produced both α and β conidia. Florida isolates produced only α conidia on OMA. The presence of all three types of conidia in pycnidia produced on plant tissue was confirmed. The remaining isolates, BG, FP1, FP3, 72A, 72B, and 75A, produced only α conidia on all of the media tested. Only the isolate ATCC 74226 produced pycnidia at regular intervals in concentric rings, rather than distributed throughout the plate (FIG. 2). All isolates produced mycelium in PDB, and produced pycnidia only at the liquid-air interface along the sides of the glass flasks. Growth rates of Florida isolate B (ATCC 74226) on the media tested are as follows: 1.9 cm/d on corn meal agar, no pycnidia produced after 30 d; 2.2 cm/d on AIA, pycnidia produced in 7 d; 2.4 cm/d on PDA, pycnidia produced in 14 d; 2.6 cm/d on V8 agar, pycnidia produced in 6 d; 2.7 cm/d on AIAS, no pycnidia produced; 3.3 cm/d on OMA, pycnidia produced in 6 d; 3.7 cm/d on OMAII, pycnidia produced in 5 d.

DNA amplification and sequence analysis.—Comparisons were performed to determine the relationship of the Florida isolate with other members of the genus *Phomopsis,* as well as selected isolates of related genera. Length variations within the sequences of the ITS regions of the chosen isolates were observed. The isolates tested had ITS1 sizes ranging from 140–175 bp. Sequences of the ITS2 region ranged in size from 144–157 bp in this region. The sequences of the isolates in this study had base composition which ranged from 45–56% GC in ITS1 and 49–59% in ITS2. Initial heuristic searches on 24 sequences found 53 equally parsimonious trees of length 251, with a consistency index (CI) of 0.946 and a retention index (RI) of 0.974 (FIG. 6). Analysis was repeated by separating the two regions into character subsets. Heuristic searches based on the sequences of ITS1 were performed with a character matrix of 298 sites in 24 isolates. This search found 52 equally parsimonious trees with a length of 154 steps, a CI of 0.799, and RI of 0.930 (FIG. 7). Heuristic searches based on the sequences of the ITS2 region were performed using a data matrix of 245 sites in 24 isolates. This search resulted in nine equally parsimonious trees of 102 steps, CI of 0.709, and a RI of 0.894.

DISCUSSION

There have been more than 400 taxa described within the genus *Phomopsis,* with no recent revision of the members of the genus. The general morphological characters of the Florida isolate B (ATCC 74226) indicate that it belongs in the genus *Phomopsis.* It shares the characteristic conidial types produced by

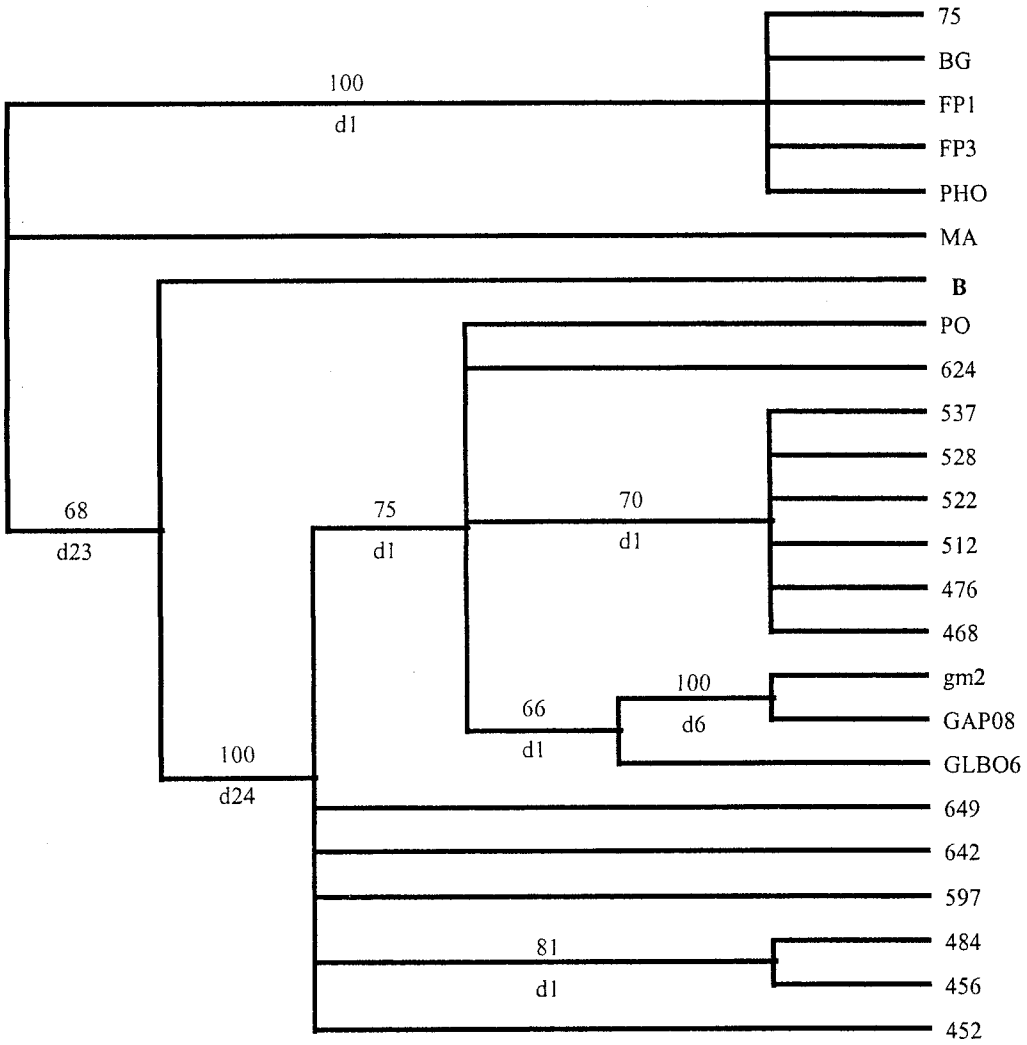


FIG. 6. Phylogenetic relationships among strains of *Phomopsis* spp. from various hosts and isolates obtained from species of *Amaranthus* based on internal transcribed spacer region (ITS) sequences. Strict consensus of 53 equally parsimonious trees (tree length = 251, consistency index = 0.946, homoplasy index = 0.054, retention index = 0.974, rescaled retention index = 0.921). Bootstrap replication frequencies greater than 50% are indicated above branches. Values below branches, preceded by a lowercase d represent decay indices values. Isolate information is listed in TABLE I.

other species of *Phomopsis* and produces these conidia in pycnidial conidiomata. The conidiophores were branched or straight and these are shared characteristics.

Determination of speciation within the genus *Phomopsis* has been highly dependent upon the host from which the isolate was described. More recent studies with several species have shown that the host ranges of many of the isolates that have been named as species are more broad than originally hypothesized, and it also has been found that a single host plant is capable of supporting more than one species. This somewhat complicates the process of speciation in this genus. The lack of a true lectotype species for the genus *Phomopsis* (Sutton 1980) further complicates taxon identification in the group.

Several characters of the Florida isolate B (ATCC 74226), named here as *P. amaranthicola,* are unique in comparison to many members of the genus. The presence of a third type of conidium, the C conidium, is reported for only a few species (Punithalingam 1975). Multiple loci of conidiogenesis are reported for no other species in the genus.

Alpha conidia are apparently the infectious propagules of this isolate and were found to germinate efficiently and to be produced abundantly on several types of media. Alpha conidia and beta conidia are commonly produced for many species of *Phomopsis* and the alpha conidia have been found to be the most predominant type. The presence of the C conidium has been reported for only three other species (Punithalingam 1975). The species *P. amaranthi*

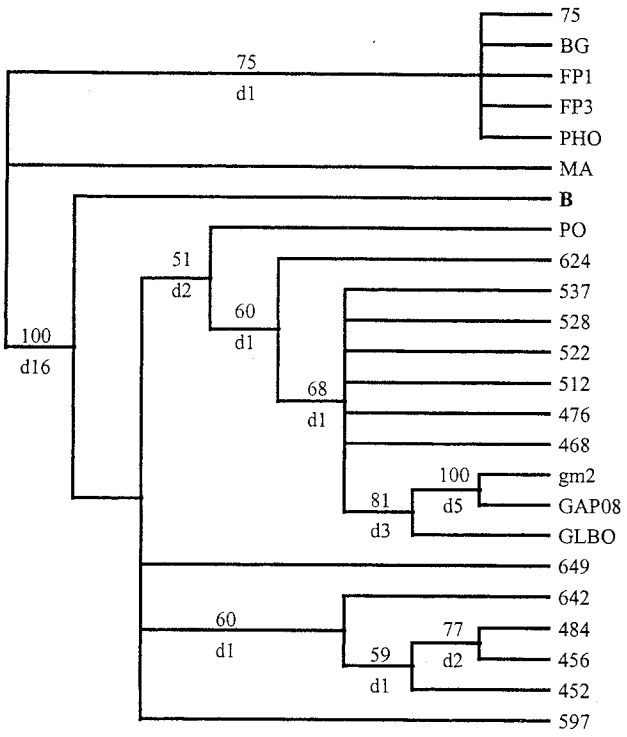


FIG. 7. Phylogenetic relationships among strains of *Phomopsis* spp. from various hosts and isolates obtained from species of *Amaranthus* based on rDNA internal transcribed spacer region 1 (ITS1) sequences. Strict consensus of 52 equally parsimonious trees (tree length = 154, consistency index = 0.799, homoplasy index = 0.201, retention index = 0.930, rescaled retention index = 0.742). Bootstrap replication frequencies greater than 50% are indicated above branches. Values below branches, preceded by a lowercase d represent decay index values. Isolate information is listed in TABLE I.

was not reported to have C conidia, nor was it reported that there were multiple loci of conidiogenesis for α conidium production. Type specimens of this species could not be obtained.

Analysis of the sequence data from a variety of isolates from *Amaranthus* spp. and other species of *Phomopsis* showed considerable variation in the ITS1 region. Nearly all nodes resolved in the strict consensus cladograms were present and received moderate to strong support by the bootstrap analyses. In all cases, the groupings resulted in the Florida isolate B (ATCC 74226), *P. amaranthicola,* falling as the sister group to the major clade containing the *Phomopsis* spp. sequenced by Rehner and Uecker (1994). The latter were grouped in this study similarly to Rehner and Uecker's groupings in their original work. The Florida isolate B (ATCC 74226), although grouping as the sister group to the other *Phomopsis* "species," relative to the *Phoma*-like isolates, was consistently outside of both of the major clades (FIGS. 6, 7).

The cladogram based on the ITS2 sequence data was less resolved than all other trees (data not shown). Cladograms that were based on sequence groups that excluded the *Phomopsis* "species" sequenced by Rehner and Uecker (1994), resulted in the grouping of the Florida isolate B as the well-resolved sister taxon within a clade containing *P. oryzae* and the *Phomopsis* spp. isolates from Georgia peach. The Florida isolate B is clearly distinct from the two major clades containing the PHO isolate and the PO isolate (FIGS. 6, 7).

A number of points complicate making conclusions about the generic placement of the named isolate. There was a high degree of variability in the sequences of the included isolates. This made alignment of the sequences difficult and somewhat ambiguous in terms of identifying the optimal alignment. Alignments are often difficult when the paired sequences differ by more than 30% (Hillis and Dixon 1991). In addition, due to the complicated taxonomic situation regarding the genus *Phomopsis,* it is impossible to determine if the terminal clades represent species or groupings of isolates that have been referred to as species. Although the Florida isolate B consistently occurs as the sister group to the remaining isolates of *Phomopsis* spp., it is difficult to determine if it belongs in this genus. The results of the sequence analysis, coupled with the unique morphological characteristics, indicate that it is not any of the existing species that have been sequenced, nor is it similar morphologically to other species of *Phomopsis* known to occur on amaranth. Therefore, it is necessary and justified to name it as a new species.

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